NOAA-NATIONAL OCEAN SERVICE

CENTER FOR COASTAL ENVIRONMENTAL HEALTH AND BIOMOLECULAR RESEARCH

FY98 SIGNIFICANT ACCOMPLISHMENTS

MARINE BIOTOXINS PROGRAM

GROWTH CONTROL OF HARMFUL ALGAL BLOOMS

Research on the biochemical pathways that control growth of red tide algae provides a new means to understand the processes that initiate harmful algal blooms and to evaluate measures to control growth of harmful algae. These pathways are amenable to chemical and biological intervention, such as that applied to inhibit growth of terrestrial plants. Current research efforts focus on the Florida red tide dinoflagellate, *Gymnodinium breve,* and the ciguatera associated dinoflagellates. Diel phasing of the cell cycle has been characterized in both laboratory cultures and field populations of the Florida red tide dinoflagellate, and the light dependent cues that couple the cell cycle to the diel cycle have been identified. The molecular regulators of the cell cycle have been shown to be sensitive to inhibition by a drug developed to inhibit growth of cancer cells. Studies on allelochemical interactions within the ciguatera dinoflagellate assemblage have identified a novel growth inhibitory compound produced by *Prorocentrum lima* and active against other dinoflagellates. Liquid chromatography-mass spectrometry has determined that this compound is unrelated to okadaic acid, the biotoxin produced by *P. lima*. Structural characterization and mode of action of this compound are currently being addressed with LC-MS and nmr.

BIOLOGICAL CONTROL MEASURES FOR HARMFUL ALGAL BLOOMS

Research on the interaction of bacteria and red tide algae has provided a new means to understand microbial processes leading to the termination of harmful algal blooms. Clones of algicidal bacteria have been isolated from both bloom and non-bloom waters in the Gulf of Mexico, and one strain from each water type is able to terminate the growth of red tide algae in laboratory cultures. The algicidal activity of one bacterium has been partially characterized, and shown to correspond to a dissolved component which exhibits a degree of taxonomic specificity in its effect on target species. Defining the role of algicidal bacteria in algal bloom termination provides a basis for new generation management efforts necessary to control harmful algal blooms.

CHARACTERIZATION OF TOXINS PRODUCED BY *PFIESTERIA PISCICIDA*

A water-soluble toxic activity produced by *Pfiesteria piscidi*a has been purified and structural analysis is underway in collaboration with North Carolina State University and the Centers for Disease Control and Prevention. Reporter gene assays and calcium digital image analysis have been used to investigate the mechanism of action of the putative toxin on calcium signaling pathways in pituitary tumor cells. The putative toxin has also been found to have comparable effects on primary cultures of neurons isolated from memory processing region of the brain (hippocampus) of laboratory rats. Partially purified preparations of this water soluble toxin have also shown to cause learning-deficits in rats by colleagues at Duke University.

DEFINITIVE CHEMICAL ANALYSIS OF BREVETOXIN IN WEST INDIAN MANATEES

Many of the unusual marine mammal mortality events attributed to harmful algal blooms remain circumstantial. The highly publicized mortality of the West Indian manatees on the west Florida shore lacked chemical confirmation of brevetoxin exposure. Methodologies to obtain ultra low limits of detection and quantification values for brevetoxin have been achieved with APSI-mass spectrometry. First time blubber has been successfully analyzed opening a new potential of life history studies in manatees and/or other marine mammals. The very low detection limits afforded by LCMS coupled to the resolving power of LC as a MS front end has allowed this otherwise very difficult matrix to be analyzed. Laboratory training on the use of this methodology was provided this summer as part of a veterinary internship program.

IDENTIFICATION OF DOMOIC ACID IN CALIFORNIA SEA LIONS AND THEIR FOODWEB

Through the combined use of rRNA probes for *Pseudo-nitzschia* spp., a receptor binding assay and a newly developed liquid chromatography-mass spectrometry method to definitively identify domoic acid, a toxic bloom of *Pseudo-nitzschia australis* occurring this past spring in Monterey Bay, CA was tracked throughout the event. Concurrent use of these novel detection methods, a collaborative effort with the Monterey Bay Aquarium Research Institute, comprises a powerful approach to acquiring near real time data for harmful algal blooms. A time course for the bloom and associated toxin levels was developed, which showed temporal changes in bloom toxicity and the subsequent appearance of a non-toxic species, *Pseudo-nitzschia pseudodelicatissima*, along with a concomitant decline in DA levels. In addition, the *P. australis* bloom was demonstrated to be the source of domoic acid transmitted through planktivorous fish (e.g., anchovies) and ultimately to sea lions in the region, which suffered a mortality event in excess of 50 animals. The latter was the first documentation of naturally incurred DA in mammalian body fluids, providing compelling evidence that the cause of sea lion mortalities was due to DA poisoning.

IMPACT OF CIGUATOXINS ON LARVAL SURVIVABILITY IN FINFISH

A new approach to model natural and anthropogenic stresses on fisheries impacted by harmful algal blooms has begun to quantify the adverse effects of cigutoxins on larval survivability. The transfer of ciguatoxin from maternal stores to the embryo have been modeled by microinjection of ciguatoxin into the yolk sac of fertilized medakafish (*Oryzias latipis*) embryos. Embryos microinjected with subpicogram quantities (0.1-0.9 pg/egg (ppb)) of ciguatoxin exhibit cardiovascular, muscular, and skeletal abnormalities and those injected with higher levels (1.0-9.0 pg/egg) exhibit significantly reduced hatchability. The concentrations of ciguatoxin observed to induce developmental toxicity are less than the amount of ciguatoxin in the flesh of fish (1 ppb) that are minimally toxic to humans and thus are relevant natural levels of exposure. The sensitivity of embryonic finfish to direct oocyte exposure indicates that maternal transfer of low levels of ciguatoxin may represent an unrecognized stress to the population dynamics of economically important reef fish and a previously undetected ecological consequence of proliferation of ciguatoxin-producing algae.